The influence of water quality and macroinvertebrate colonization on the breakdown process of native and exotic leaf types in sub-alpine stream

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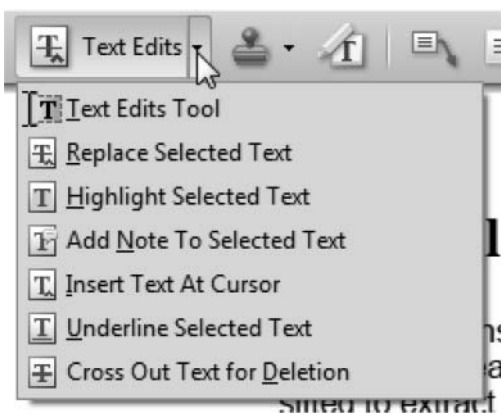

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The influence of water quality and macroinvertebrate colonization on the breakdown process of native and exotic leaf types in sub-alpine stream

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Most of the energy input of low-order lotic food webs derives from non-living sources of terrestrial organic matter. For this reason, many studies have examined patterns of leaf breakdown; most recently, interest has focused on the importance of water quality or the nature (native versus exotic) of plant material. In this study, we combined both aspects by analyzing the breakdown process and macroinvertebrate colonization of leaf bags containing leaves of different plant types in two nearby sites with different levels of water quality. We exposed a total of 600 leaf bags made of five leaf types (three native: *Alnus incana*, *Populus alba* and *Quercus robur*; and two exotic: *Reynoutria japonica* and *Robinia pseudoacacia*) at two sites of the Pellice River (northwestern Italy). Leaf bags were retrieved after 10, 20, 30, 40, 50 and 60 days, leaf mass loss determined and the associated macroinvertebrates quantified. Significant differences were found in the mass loss and in the colonization of leaf bags between sites but not between native and exotic species. Dry mass loss was different among species but without any evident relation with exotic or native origin of plants. In our study sites, geographical origin of plant detritus is not per se central in shaping macroinvertebrate colonization and mass loss because the impact of wastewater treatment plant effluent seems to be much more important than plant origin in the breakdown process.

Keywords: macroinvertebrates; water quality; leaf bag; exotic riparian vegetation; Pellice River

Introduction

The importance of allochthonous organic matter inputs in stream food webs was early recognized and since the seminal work of Petersen and Cummins (1974), this topic has become a central subject of research in stream ecology (Vannote et al. 1980; see review in Tank et al. 2010). Leaf litter input from riparian vegetation represents an important source of energy, especially to low- and medium-order lotic environments (Gessner et al. 1999; Sabater et al. 2008), where current velocity and shading limit the opportunities for photosynthesis (Wallace et al. 1997). Terrestrial leaves that enter the stream generally breakdown through a sequence of processes (Webster & Benfield 1986), such as leaching (dissolution of labile organic compounds), conditioning (microbial colonization by bacteria and

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hyphomycetes), and consumption and fragmentation by macroinvertebrates and physical abrasion (Tank et al. 2010). The rate of leaf litter breakdown is determined directly by intrinsic characteristics of the debris (LeRoy & Marks 2006; Lecerf & Chauvet 2008), water quality and geomorphological characteristics (Suberkropp & Chauvet 1995; Sponseller & Benfield 2001), and indirectly through the activity of microbial and macrobenthic organisms responsible for conditioning, fragmentation and consumption (Cuffney et al. 1990; Findlay 2010). Breakdown of allochthonous leaf litter is generally measured using the leaf bag approach, because these bags are generally thought to be representative of the natural leaf accumulation on stream beds (see review in Graça et al. 2005).

Because of the great importance of allochthonous leaves in low-order streams (Hieber & Gessner 2002), any disturbance that results in a change in the quality or amount of allochthonous organic inputs might alter stream energy budgets and ecosystem functioning. In the last decades, lotic and riparian ecosystems have experienced severe degradation in many countries, because of increased water quality alteration (Mason 2002; Dudgeon et al. 2006) and the substitution of native forest with exotic riparian plant species (Richardson et al. 2007).

Leaf breakdown is generally considered a good indicator of stream integrity, especially because it accounts for a variety of biological, chemical and physical conditions (Niyogi et al. 2003; Elosegi et al. 2006; Woodward et al. 2012). Numerous studies have investigated the relationship between leaf breakdown processes and water quality (Young et al. 2008). In addition, some recent studies have investigated the impact of the widespread introduction of exotic plant species on the dynamics of litter in streams (Ferreira et al. 2006). Nowadays, as a result of direct introduction by humans, hydrological disturbance and natural connectivity of lotic environments, exotic plant species represent an average of more than 20% of plant species of riparian areas (Richardson et al. 2007). In fact, this topic is quite complex, as exotic species have little effect on leaf decomposition patterns if their physical and chemical characteristics are similar to the native species (Braatne et al. 2007), but differences in decomposition rate tend to emerge where exotics are invasive species with different leaf toughness (Serra et al. 2013) or C:N ratio (Royer et al. 1999). In general, many studies suggested that the presence of exotic plant species could modify the availability and turnover of benthic leaves, influencing the whole ecosystem efficiency (Pozo et al. 1997).

These perturbations (water quality alteration and invasion of exotic species) might occur together and it is likely that both of them will become more important in the future due to intensification of human activities and climate change effects. Therefore, it is necessary to address the effect of both changes in combination. In this study, we hypothesize that mass loss and colonization of leaf bags by macroinvertebrates can be influenced by both the water quality and the type of leaf material. Research conducted so far in this context has focused only on one of these effects. The purpose of this study is to combine both impacts, analyzing the breakdown process of native and exotic terrestrial leaf materials in lotic reaches with different water quality levels.

Methods

The study took place in two sites of the Pellice River, a third-order, sub-alpine, open-canopy stream in northwest Italy: site A (Villar Pellice – 44°80′45.9″ N, 7°15′74.0″ E) and site B (Luserna – 44°80′86.5″ N 7°27′35.7″ E). Sites are relatively close to one another (about 9 km), with no relevant differences in morphology, discharge or flow velocity. At both the sites, the channel width was about 7–9 m, with a mean depth of 40–60 cm.

Stream substrate was generally coarse, consisting mainly of gravel, cobbles and boulders. The climate is temperate alpine, with high autumn rainfall and spring snowmelt. Sites differ in water quality because the downstream site (B) receives wastewater treatment plant effluent; this originates from the municipal wastewater treatment plant (sedimentation and aerobic digestion only; at present decommissioned) of the city of Luserna (approximately 5000 inhabitants). The plant contributed approximately 10% of the total stream discharge. In late autumn, when leaves detach and fall from trees naturally and accumulate in the stream, we placed 300 leaf packs in the river bed at each site consisting of five leaf types, two exotic (Japanese knotweed *Reynoutria japonica* Houtt and black locust *Robinia pseudoacacia* L.) and three native (Grey Alder *Alnus incana* Moench, white poplar *Populus alba* L. and pedunculate oak *Quercus robur* L.). These species were chosen because they were common in the riparian zone of the Pellice River and all contribute to the benthic litter standing stock. Leaves were collected *in situ* upon natural abscission, then they were air dried. Approximately 5.03 g (±0.04 SD) of air-dried leaves were placed in nylon mesh bags (0.5 cm mesh size), after having been weighed to the nearest 0.01 g and then humidified to minimize breakage, according to Woodcock and Huryn (2005). We used this mesh size to allow access by macroinvertebrates that utilize leaves and associated matter as food and shelter. On 9 December 2010, 60 bags/species were placed in each site. Packs were fixed to stones and randomly located in riffle areas. After 10, 20, 30, 40, 50 and 60 days, 10 bags of each leaf species were removed from each site, inserted separately into plastic bags with stream water and immediately transported to the laboratory. Macroinvertebrates were sorted under a dissecting microscope (Nikon® SMZ-1500). All macroinvertebrates were manually collected with forceps and preserved in 70% ethanol. Organisms were counted and identified to the genus level, except for Chironomidae, Simuliidae and early instars of some Trichoptera and Diptera, which were identified to the family level. Leaves were washed to remove silt, and then oven dried at 105 °C until a constant mass was reached in order to determine the remaining mass. Leaf bag mass loss was quantitatively modeled using percentage remaining mass, and the breakdown rate (k) was then calculated by using an exponential decay model with the scope to incorporate all of the temporal variations (Benfield 1996; Bärlocher 2005).

Main chemical and microbiological parameters at the two stations were collected at the beginning of the study, by applying the following standard tests: 2030, 4110A2, 4020, 3030, 7030 MAN 29/2003 and ISO 157052002. Water temperatures were measured hourly in the two sites with HOBO® Water Temp Pro Dataloggers (0.01 °C accuracy).

To compare mass loss, we performed a two-way ANOVA using the factors site and species. We used remaining mass as the dependent variable, separately for each removal dates (10, 20, 30, 40, 50 and 60 days). We tested normality using the Kolmogorov–Smirnov test and homoscedasticity using the Levene's test (Levene 1960). As data had no homoscedasticity in the 60 days removal date, we performed an x^2 transformation. After this, all data followed a normal distribution (K-S, $p > 0.05$) and had homogeneity of variances (Levene's test, $p > 0.05$). To detect eventual differences between sites in macroinvertebrate abundance (N) and taxa richness (S), we performed a Mann–Whitney U-test for each leaf species on the six removal dates separately, as data did not follow a normal distribution. On the fourth removal date, when, according to previous studies, assemblage of colonizer macroinvertebrates is sufficiently structured (Peralta-Maraver et al. 2011), we also performed an ANOSIM test (with 10,000 permutations) comparing community composition between sites (considering only this factor) and one ANOSIM test (also with 10,000 permutations) for each site separately comparing community composition with leaf species as a factor.

Table 1. Main chemical and biological variables at the two sites of the Pellice River at the beginning of the experiment (extended biotic index, Ghetti, 1997).

Parameters	Site A	Site B
Conductivity (μS/cm)	142	516
DO (mg/L)	9.0	6.5
Total P (mg/L)	<0.05	1.13
COD (mg/L)	<5	42.3
NH_4^+ (mg/L)	<0.05	4
NO_3^- (mg/L)	3	4.2
pH	6.90	7.78
Escherichia coli (CFU/mL)	35	2330
Extended biotic index value	10	5
Extended biotic index class	I	IV

Results

We detected an evident difference in the water quality between the two sites, because in site B the wastewater treatment plant effluent caused an increase in organic matter, temperature, nutrient concentrations and conductivity, and a decrease in dissolved oxygen levels (Table 1; Figure 1). The biological quality, described by the extended biotic index (IBE, Ghetti 1997), dropped from the first (at site A) to the fourth class (at site B, Table 1).

Leaf bags exhibited an exponential mass loss over time (Figure 2). We detected significant differences in the mass loss among leaf species and also between sites (Table 2). We found significant differences among species in mass loss, but these differences were not related to the plant type. Comparing patterns in the two sites, we detected that two native species showed the most diverse trends of mass loss. *P. alba* showed the highest

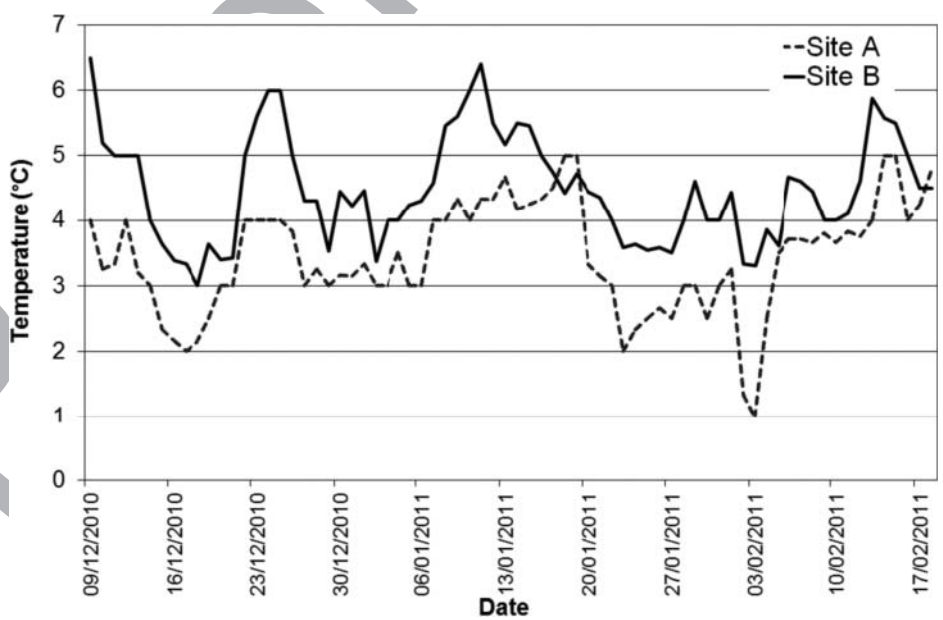

Figure 1. Water temperatures in the two study sites of Pellice River.

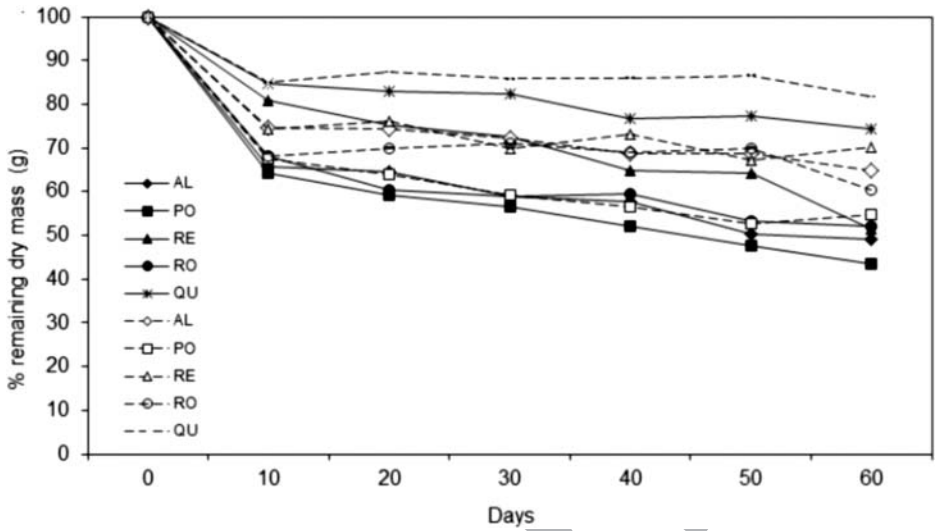

Figure 2. Mean remaining mass in leaf bags on the six removal dates (mean ± SE; solid symbols = unpolluted site A; open symbols = polluted site B; RE = *Reynoutria japonica*, RO = *Robinia pseudoacacia*; AL = *Alnus incana*, PO = *Populus alba* and QU = *Quercus robur*).

Table 2. Two-way ANOVA results of leaf mass remaining using as factors site and leaf species. A separate analysis was conducted for each harvest date.

	df	SS	MS	*F*	*p*
10 days					
Site	1	0.096	0.096	0.89	<**0.001**
Leaf species	4	10.4	2.60	24.2	<**0.001**
Site*Leaf species	4	1.56	0.38	3.62	<**0.01**
Error	90	9.67	0.11		
Total	99	21.7			
20 days					
Site	1	2.13	2.13	26.1	<**0.001**
Leaf species	4	17.3	4.33	53.0	<**0.001**
Site*Leaf species	4	0.71	0.17	2.17	n.s.
Error	90	7.36	0.081		
Total	99	27.5			
30 days					
Site	1	2.06	2.06	28.7	<**0.001**
Leaf species	4	19.2	4.80	66.7	<**0.001**
Site*Leaf species	4	2.27	0.56	7.88	<**0.001**
Error	89	6.41	0.07		
Total	98	29.8			
40 days					
Site	1	3.65	3.65	21.6	<**0.001**
Leaf species	4	16.5	4.12	24.3	<**0.001**
Site*Leaf species	4	0.45	0.11	0.66	n.s.

(*continued*)

Table 2. (*Continued*).

	df	SS	MS	*F*	*p*
Error	91	15.4	0.17		
Total	100	36.1			
50 days					
Site	1	6.38	6.38	80.78	<**0.001**
Leaf species	4	26.78	6.69	84.75	<**0.001**
Site*Leaf species	4	2.10	0.53	6.67	<**0.001**
Error	87	6.84	0.079		
Total	96	42.23			
60 days					
Site	1	9.18	9.18	50.70	<**0.001**
Leaf species	4	23.00	5.75	31.76	<**0.001**
Site*Leaf species	4	1.18	0.30	1.63	n.s.
Error	90	16.30	0.18		
Total	99	49.66			

mass loss, while *Q. robur* the lowest in both A and B sites, while the native *A. incana* and the exotic *R. japonica* and *R. pseudoacacia* showed intermediate patterns (Figure 2). Furthermore, leaf breakdown appeared to proceed faster at the control site (A) compared to the polluted site (B). Values of breakdown rates (*k*) are reported in Table 3.

Regarding the macroinvertebrates colonizing leaf bags, we generally found higher abundances and higher taxonomic richness at the unpolluted site A than in the polluted site B (Figure 3(a) and 3(b)). Assemblages of colonizing benthic macroinvertebrates after 40 days were clearly different between sites. In the leaf bags of the unpolluted site A, Plecoptera represented 73.4% of total collected invertebrates, with *Leuctra* spp. and *Nemoura* spp. being the most abundant taxa. Diptera, Ephemeroptera and Trichoptera were less abundant, accounting, respectively, for 17.7%, 6.28% and 2.45% of total invertebrates. Oligochaeta, Crustacea and Tricladida together accounted for only 1.7% of the total. On the contrary, colonizing assemblages in the site B were dominated by Diptera (81.1% of total, mostly represented by Chironomidae, 56.1%). Also, Annelida were conspicuously represented, with Oligochaeta (9.56%, mostly Naididae and Tubificidae) and Hirudinea (8.08%, principally *Erpobdella* sp.). Plecoptera, Ephemeroptera and Trichoptera were almost exclusively absent in the B samples (Table 4). Regarding the macroinvertebrate colonizing assemblages, we noticed some evident temporal differences between the two sites: in site A we observed, in general, a trend of positive growth while

Table 3. Species-specific decomposition rates (*k*, d^{-1}) for the different leaf types at the two sites.

Site	A	B
AL	0.012	0.007
PO	0.014	0.010
RE	0.011	0.006
RO	0.011	0.008
QU	0.005	0.003

RE = *Reynoutria japonica* and RO = *Robinia pseudoacacia*; AL = *Alnus incana*, PO = *Populus alba* and QU = *Quercus robur*.

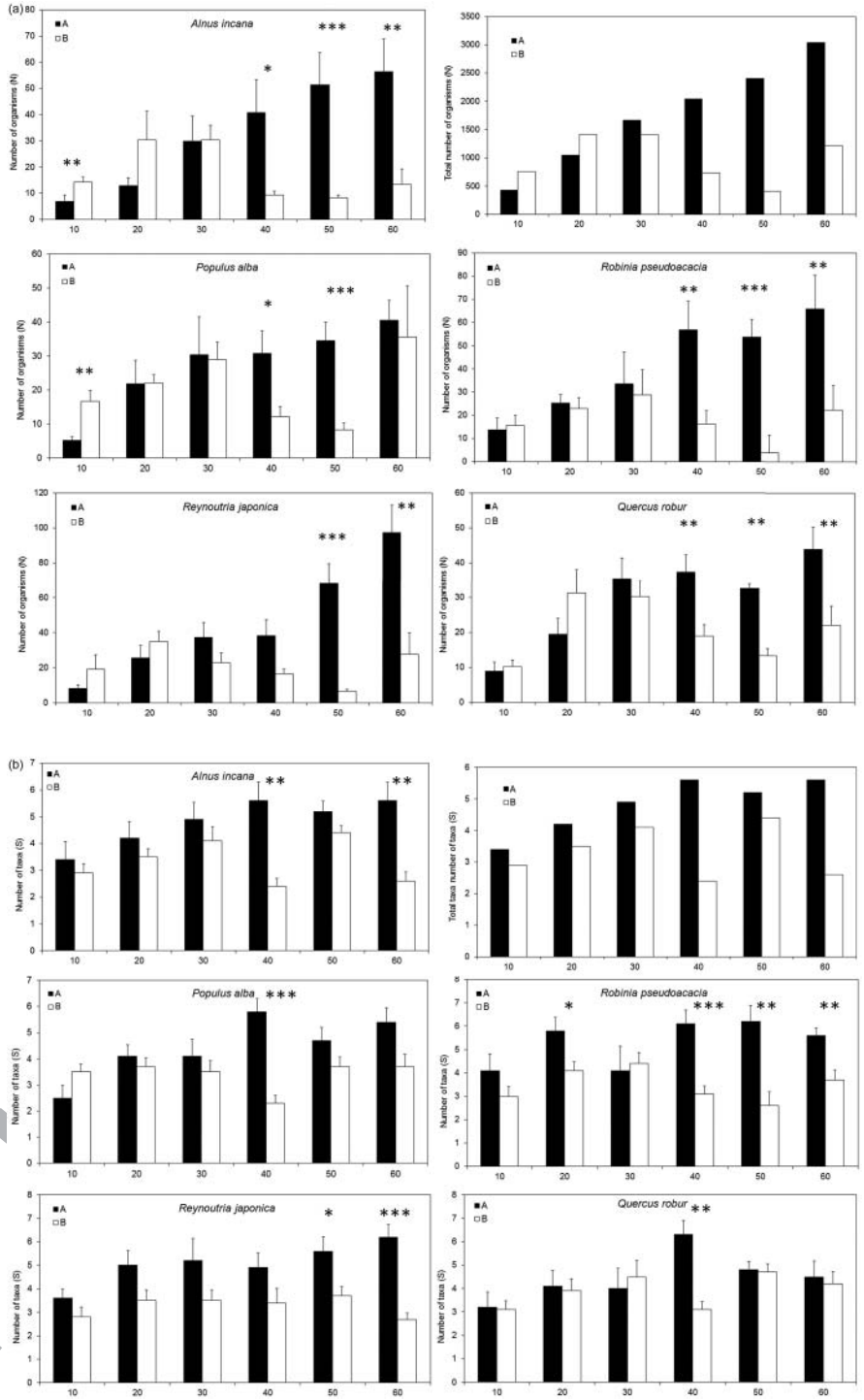

Figure 3. Number of (a) invertebrates and (b) taxa colonizing leaf bags during the study period (solid symbols = unpolluted site A; open symbols = polluted site B).

Table 4. Total number of individuals of the most dominant taxa at each site.

Taxa		Site A	Site B
Leuctridae	*Leuctra* sp.	6099	1
Nemouridae	*Nemoura* sp.	1113	0
	Amphinemura sp.	199	0
Perlodidae	*Isoperla* sp.	370	0
Heptageniidae	*Ecdyonurus* sp.	565	1
	Rhithrogena sp.	55	0
Limnephilidae		205	0
Hydropsychidae	*Hydropsyche* sp.	30	35
Chironomidae		1734	3327
Athericidae	*Atherix* sp.	104	15
Psychodidae		7	163
Erpobdellidae		0	478
Naididae		1	240
Tubificidae		0	152
Lumbriculidae		0	135
Lumbricidae		1	40

in site B we normally detected an intermediate peak. This happened, generally speaking, for both macroinvertebrate abundance (N, Figure 3(a)) and taxa richness (S, Figure 3(b)). The ANOSIM test showed a significant difference between sites in the community composition when considering all leaf species together (ANOSIM $R = 0.62$, $p < 0.05$), but no significant differences within each site among leaf species (ANOSIM $R = 0.02$, $p > 0.05$ for site A and ANOSIM $R = 0.01$, $p > 0.05$ for site B).

Discussion

Litter inputs from the riparian vegetation are of fundamental importance in low-order stream systems, where they represent the main energy source and support complex benthic food webs (Tank et al. 2010). For this reason, it is crucial to investigate the effects of human activities on this process. In this study, we analyzed the breakdown process of terrestrial litter inputs by comparing (1) the type (native/exotic) of the leaf litter and (2) the level of water quality (unpolluted/polluted) of the stream reach. In the last several decades, there has been an impressive increase in the number and spread of exotic, invasive plant species in riparian environments (Richardson et al. 2007). Some studies suggested that invasive species can cause alterations at different levels in the structure and functioning of ecosystems (Ehrenfeld 2010). Moreover, the establishment of exotic riparian plants can alter the exchange of organic material between terrestrial and lotic ecosystems, both changing the quantity (Mineau et al. 2011, 2012) or the quality (Bärlocher & Graça 2002) of terrestrial litter inputs, leading to alterations in the composition (Serra et al. 2013) and density (Lester et al. 1994) of benthic assemblages. Many studies have shown that, for instance, the introduction and spread of *Eucalyptus globulus* plantations in Spain had a strong effect on in-stream terrestrial detritus colonization and degradation (Chauvet et al. 1997; Pozo et al. 1998; Ferreira et al. 2006), while other studies have shown weak influence of exotic species on streams (Braatne et al. 2007).

Our study gave evidence that mass loss of leaf bags was generally higher in the unpolluted site A rather than in the polluted site B. Comparing the breakdown rate (k) of the five leaf species, the highest values were always found in the unpolluted site A. *P. alba*,

R. japonica, *R. pseudoacacia and A. incana* showed a 'fast' decay in site A and a 'medium' decay in site B (*sensu*, Bärlocher 2005). Although the decay of *Q. robur* was always classified as 'slow', it was faster in site A than in site B. Interestingly, we observed that decomposition rates were different among species and, therefore, organic litter might be available at different times within the system. The two exotic species exhibited breakdown rates that were similar to *A. incana* but lower than those of *P. alba* and faster than those of *Q. robur*. If the non-native plant species are invasive, and therefore, create the majority of the detritus available within the system; this may compound the effects of the poor water quality on the stream ecological function. We must consider that many invasive species are good competitors and may form monospecific populations in riparian areas. This could result in the limited availability of allochthonous organic input into the river system with potentially serious repercussions throughout the lotic system.

The ANOSIM test showed clear differences in macroinvertebrate assemblages among sites. In the unpolluted site A, bags were colonized by very diverse macroinvertebrate assemblages, with an important presence of stenoecious taxa such as Plecoptera, some Diptera, Ephemeroptera and Trichoptera. In the polluted site B, bags were almost entirely colonized by euriecious and tolerant taxa, such as Chironomidae and Annelida. This diversity is also evident analyzing the temporal trends of colonization: while in site A the resource is colonized over time by a growing number of individuals and taxa, in site B an intermediate (and lower) peak is reached and then macroinvertebrate assemblages began to diminish in both density and taxonomic richness. The difference in mass loss could be related to these differences in colonizing assemblages, because it is known that a diminution in the diversity, including both taxonomic and functional aspects, reduces ecosystem functioning (Woodcock & Huryn 2005). Shredders, such as Leuctridae, Nemouridae and Limnephilidae, constitute the largest colonizing group in site A, while they are almost absent in site B, where macroinvertebrate assemblages were dominated by collectors–gatherers, such as Chironomidae and various groups of Oligochaeta (Table 4). It is known that wastewater treatment plant effluents can have significant impacts on macrobenthic communities or in-stream primary productivity (Bo & Fenoglio 2011), but this study underlines that they can also influence the decomposition rate of terrestrial organic matter. Our findings suggest that the richer benthic communities in site A increased leaf breakdown rates (despite site B having a warmer temperature; Figure 1). On the other hand, ANOSIM tests did not show any clear difference in leaf bag macroinvertebrate assemblages between native and exotic species either in site A or in site B.

Our study supports the hypothesis that geographical origin of plant detritus (native/exotic) is not per se central in shaping macroinvertebrate colonization and mass loss in the Pellice River. The main result of this study is that, considering both mass loss and colonizing macroinvertebrate characteristics, the most significant impact was related to the water quality change due to the wastewater treatment plant effluent in site B. We can conclude that the effect of exotic terrestrial organic input on in-stream processes can differ widely, depending on the nature of the material but that the origin of the debris may be less important than water quality.

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